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Review

# The stability of blood fatty acids during storage and potential mechanisms of degradation: A review

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Fatty acids in blood samples, particularly polyunsaturated fatty acids (PUFAs), are susceptible to degradation through peroxidation reactions during long-term storage. Storage of blood samples is necessary in almost all studies and is crucial for larger clinical studies and in field research settings where it is not plausible for analytical infrastructure. Despite this, PUFA stability during blood storage is often overlooked. This review introduces and discusses lipid peroxidation and popular strategies employed to prevent or minimize peroxidation reactions during fatty acid analysis. Further, an in-depth examination of fatty acid stability during storage of blood is discussed in detail for all blood fractions including plasma/serum, erythrocytes and whole blood stored both in cryovials and on chromatography paper before discussing the associated mechanisms of degradation during storage. To our knowledge this is the first review of its kind and will provide researchers with the necessary information to confidently store blood samples for fatty acid analysis.

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## 1. Introduction

The use of omega-3 blood biomarkers in clinical studies has increased dramatically since 2004 with the proposal of the sum of the relative percentage of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) in erythrocytes as a potential risk factor for coronary heart disease [1], and the development of rapid fingertip prick (FTP) capillary blood collection techniques for fatty acid profiling [2]. EPA+DHA in erythrocytes (also known as the “Omega-3 index”) and other fatty

*Abbreviations:* FTP, fingertip prick; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HUFA, highly unsaturated fatty acid; PUFA, polyunsaturated fatty acid; ARA, arachidonic acid; Hb, hemoglobin; BHT, butylated hydroxytoluene; PL, phospholipid; TAG, triacylglycerol; CE, cholesteryl ester; NEFA, non-esterified fatty acid; LNA, linoleic acid; PLA2, phospholipase A2; lyso-PC, lyso-phoshatidylcholine; TLE, total lipid extract; EDTA, ethylene diamineteraacetic acid; DBS, dried blood spots; $Fe^{2+}$, ferrous iron; $Fe^{3+}$, ferric iron

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acid blood biomarkers continue to be used to assess cardiovascular disease risk [3], as initially proposed, but also for maternal and infant health [4,5] and dietary intake assessments [6,7]. Whole blood fatty acid assessments via fingertip prick collection have become common practice due to ease of collection, minimal invasiveness and amenability to high-throughput analytical techniques, however, translational equations to estimate erythrocyte EPA+DHA equivalents [8] may still be required for health risk assessments as patterns of incorporation of EPA and DHA in blood fractions are different [9].

Improved analytical throughput via FTP blood collection can increase the reliance on temporary blood storage, such as during clinical studies that collect thousands of samples and may require years of storage prior to analysis, and during field research. Ultra-cold storage infrastructure is often limited or unavailable, particularly in the latter scenario. In addition, both clinical and field research frequently requires shipment of samples from multiple locations to specialized fatty acid assessment laboratories, which adds additional questions regarding storage stability. Although many study protocols require that blood samples are stored for long periods of time, it is often overlooked that under certain storage conditions fatty acids may be highly susceptible to degradation through lipid peroxidation.

Making fatty acid assessments and storage stability more difficult to understand and interpret is that analytical techniques employed for fatty acid determinations are variable and depend largely on the sample of interest. Plasma [10], erythrocytes [11], whole blood [12] and FTP blood [13] are all utilized for the determination of omega-3 status. Furthermore, omega-3 blood biomarkers reported in the literature are variable and fatty acid storage stability may be dependent on the specific biomarker assessed [14]. Reported omega-3 blood biomarkers include but are not limited to the percentage of % EPA+DHA [1,15], the percentage of omega-3 highly unsaturated fatty acids (HUFA) in total HUFA [16], ratio of omega-6 polyunsaturated fatty acids (PUFA) to omega-3 PUFA (n-6/n-3 ratio) [17] and ratio of EPA to arachidonic acid (ARA, 20:4n-6) [18]. In fatty acid research, and particularly with omega-3 fatty acids and blood biomarker assessments, understanding storage stability is imperative as omega-3 fatty acids contain between three to six double bonds making them highly susceptible to degradation through lipid peroxidation pathways [19].

The purpose of this review is to provide a more complete understanding of the stability of fatty acids during long-term storage in plasma, erythrocytes and whole blood stored in both cryovials and on chromatography paper, and to discuss the mechanisms of degradation and treatments available to minimize these losses during storage. Specifically, we will examine mechanisms related to fatty acid and lipid peroxidation, strategies employed to prevent fatty acid peroxidation, the present literature on sample storage in plasma, erythrocyte and whole blood fractions, and mechanisms related to fatty acid degradation during storage. Additional focus will be given to storage of whole blood collections due to their recent increase in use and the relatively few prior studies assessing storage stability in these blood fractions. Additional mechanistic focus will be placed on the relatively recent understanding that storage of these whole blood fractions in addition to erythrocytes at −20 °C results in rapid degradation in PUFA.

## 2. Lipid peroxidation

Whether bound or unbound to a glycerol or other lipid backbone molecules, PUFA are highly susceptible to peroxidation reactions as a result of the attack of free radicals. Lipid

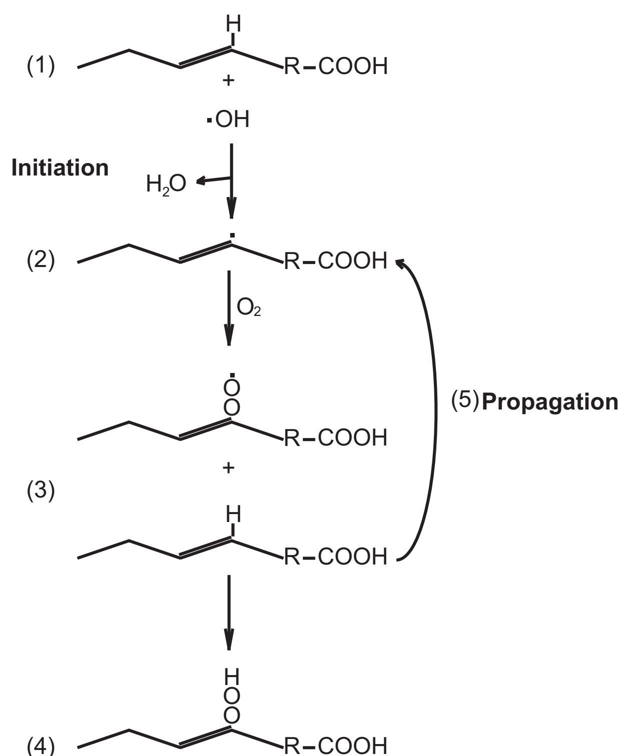


**Fig. 1.** Mechanism of free radical induced fatty acid peroxidation. (1) Unsaturated fatty acid reacts with hydroxyl radical and yields water to form (2) lipid radical which reacts with oxygen to form (3) lipid peroxyl radical that reacts with a fatty acid to form (4) lipid peroxide. The last reaction forms a new lipid radical that (5) propagates back to step (2) and re-enters the pathway.

peroxidation occurs when a highly reactive hydrogen atom is removed from the methylene group (–C=C–) of PUFA by a free radical resulting in the formation of a lipid peroxyl radical in its place [20]. This lipid peroxyl can react further with another PUFA to form the lipid peroxide in a continuous chain of lipid peroxidation reactions [19] (Fig. 1). However, lipid peroxidation reactions slow down when the ratio of proteins to fatty acids becomes high, in which case proteins become more susceptible to free radical attack [19].

A free radical is any molecule that contains an unpaired electron such as low activity molecules like the superoxide radical (•$O_2$) and highly reactive molecules such as the hydroxyl radical (•OH) [21]. The greater the number of double bonds present in a fatty acid, the greater the reactivity of the free radical to the reactive hydrogens on the methylene groups, thus explaining why fatty acids with more double bonds degrade at a faster rate than monounsaturated (one double bond) or saturated fatty acids (no double bonds) [19]. Other mechanisms of PUFA peroxidation exist, particularly in erythrocytes that make up nearly half of a whole blood sample and are relatively high in iron (Fe). In healthy cells, approximately 3% of the hemoglobin–ferrous iron complex (Hb–$Fe^{2+}$) is converted to Hb–ferric iron ($Fe^{3+}$) by $O_2$. This conversion results in the production of the •$O_2$ radical [22–24] that can subsequently attack PUFA resulting in lipid peroxidation. Additionally, $Fe^{2+}$ can accept a proton from $H_2O_2$ that results in the formation of the more potent •OH radical [19] (Fig. 2). The formation of $Fe^{3+}$ on its own is able to act directly on PUFA to accept an electron from double bonds and also yields lipid radicals [25]. Lipid radicals react with $O_2$, and the resultant lipid peroxyl radicals formed from PUFA can be converted to lipid hydroperoxides by vitamin E for removal; however, if efficient removal does not occur, then the hydroperoxides can decompose to form more free radicals in the presence of iron, thereby, further exacerbating oxidative damage

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